



**PRODUCT INFORMATION &
MANUAL**

**Mitochondrial Complex I
Activity Assay Kit
(Colorimetric)
*NBP3-25843***

For research use only.
Not for diagnostic or therapeutic
procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Novus kits are guaranteed for 6 months from date of receipt

Mitochondrial Complex I (NADH-CoQ Reductase) Activity Assay Kit

Catalog No: NBP3-25843

Method: Colorimetric method

Specification: 96T (Can detect 48 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 15.58 U/L

Detection range: 15.58-224.2 U/L

Average intra-assay CV (%): 0.8

Average inter-assay CV (%): 8.4

Average recovery rate (%): 102

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲ Intended use

This kit can measure mitochondrial complex I (NADH-CoQ Reductase) activity in animal tissue sample.

▲ Detection principle

Mitochondrial complex I catalyzes the reaction of NADH with ubiquinone substrate to generate NAD^+ and reduced ubiquinone. The specific inhibitor rotenone can inhibit the enzyme, and the total enzyme activity minus the enzyme activity after inhibitor inhibition can reflect its specific enzyme activity.

▲ Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extraction Solution A	50 mL × 2 vials	-20°C , 12 months
Reagent 2	Extraction Solution B	50 mL × 1 vial	-20°C , 12 months
Reagent 3	Protease Inhibitor	0.4 mL × 2 vials	-20°C , 12 months, shading light,
Reagent 4	Buffer Solution	15 mL×2 vials	-20°C , 12 months, shading light,
Reagent 5	Substrate A	Powder×2 vials	-20°C , 12 months, shading light,
Reagent 6	Substrate B	Powder×1 vial	-20°C , 12 months, shading light,
Reagent 7	Inhibitor	1.5 mL×2 vials	-20°C , 12 months, shading light,
Reagent 8	Negative Reagent	1.5 mL×2 vials	-20°C , 12 months, shading light,
	UV Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.

▲ Materials prepared by users

Instruments

Centrifuge, 37°C incubator, Microplate reader (340 nm)

Reagents:

Anhydrous ethanol (AR).

▲ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. During reagent preparation, it is necessary to ensure that the prepared reagent 6 working solution is completely dissolved. It is recommended to extend the time of oscillation, transfer the reagent in a EP tube to check whether the reagent is completely dissolved to clear.
2. During sample measurement, if the OD value decreases by more than 0.3 within 3 min, the sample should be diluted to ensure that the sample measurement is within the interval of uniform reaction speed. If necessary, the OD value can be measured every minute to observe its dynamics changes.
3. It is recommended to use fresh sample for detection.
4. It's better to measure no more than 8 sample wells at same time.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. **Preparation of reagent 5 working solution:**
Dissolve a vial of reagent 5 with 15 mL reagent 4 and mix fully. The prepared solution can be stored at 2-8°C with shading light for 12 h.
3. **Preparation of reagent 6 working solution:**
Dissolve a vial of reagent 6 with 4 mL anhydrous ethanol and shake until it turned yellowish clear liquid. The prepared solution can be stored at 2-8°C with shading light for 48 h.
4. **Preparation of reaction working solution:**
Mix the reagent 6 working solution and reagent 5 working solution at the ratio of 1:59 fully. Prepare the fresh needed amount before use and the prepared solution should be placed on the ice box with shading light and used within 1 h.

▲ Sample preparation

Tissue:

Accurately 0.1g weigh the tissue, then add 0.9 mL reagent 1 and 0.01 mL reagent 3 to homogenize the sample. Then centrifuge at 600 g for 5 min at 4°C, discard the precipitate and take the supernatant. Then centrifuge at 15000 g for 10 min at 4°C, discard the supernatant and take the precipitate. The precipitate was mixed with 200 µL of reagent 2 and 2 µL of reagent 3, sonicated for 1 min, centrifuged at 15000 g at 4°C for 10 min. Then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

▲ Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (15.58-224.2 U/L).

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Rat muscle tissue homogenate	1-2
10% Rat lung tissue homogenate	4-8
10% Mouse liver tissue homogenate	1-2
10% Mouse heart tissue homogenate	4-8
10% Rat heart tissue homogenate	1-2
10% Rat liver tissue homogenate	1-2
10% Rat kidney tissue homogenate	2-4
10% Porcine heart tissue homogenate	1-2

Note:The diluent is reagent 2.

Assay protocol

▲ Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'	S41	S41'
B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'	S42	S42'
C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'	S43	S43'
D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'	S44	S44'
E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'	S45	S45'
F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'	S46	S46'
G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'	S47	S47'
H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'	S48	S48'

[Note]: S1–S48, sample wells; S1'–S48', control wells.

▲ Detailed operation steps

- 1) **Control well:** Add 20 μL of sample to the corresponding wells.
Sample well: Add 20 μL of sample to the corresponding wells.
- 2) **Control well:** Add 20 μL of reagent 8 to the corresponding wells.
Sample well: Add 20 μL of reagent 7 to the corresponding wells.
- 3) Mix fully and incubate at 37°C for 3 min.
- 4) Add 200 μL of reaction working solution to each well.
- 5) Measure the OD value of each well at 340 nm with microplate reader, recorded as A_1 . 3 min later, measure the OD value of each well at 340 nm with microplate reader, recorded as A_2 , $\Delta A = A_1 - A_2$.

Note: The control wells measure the total enzyme activity, and the sample wells measure the non-specific enzyme activity. After adding the reaction working solution, record the OD value once every minute for 3 min, observe the change of OD value within 3 min to ensure whether is a constant rate of decline.

▲ Summary operation table

	Control well	Sample well
Sample (μL)	20	20
Reagent 8 (μL)	20	
Reagent 7 (μL)		20
Mix fully and incubate at 37°C for 3 min.		
Reaction working solution (μL)	200	200
Measure the OD value of each well, recorded as A_1 . 3 min later, measure the OD value of each well, recorded as A_2 , $\Delta A = A_1 - A_2$.		

▲ Calculation

Tissue ana cell:

Definition: The amount of mitochondrial complex I in 1 g tissue mitochondria protein per 1 minute that catalyze the decomposition of the 1 μmol NADH at 37°C is defined as 1 unit.

$$\begin{aligned} & \text{mitochondrial complex I activity (U/gprot)} \\ &= (\Delta A_{\text{Control}} - \Delta A_{\text{Sample}}) \div (6600 \times 0.7) \times V_1 \div T \div V_2 \div C_{\text{pr}} \times f \times 10^6 \end{aligned}$$

Note:

$\Delta A_{\text{Control}}$: The change OD value of control ($A_1 - A_2$).

ΔA_{Sample} : The change OD value of sample ($A_1 - A_2$).

6600: The molar extinction coefficient of NADH, L/(mol•cm)

0.7: Optical path, cm

V_1 : The volume of the reaction system, 0.24 mL

V_2 : The volume of the sample, 0.02 mL

T: The time of reaction, 3 min

f: Dilution factor of sample before test

C_{pr} : The concentration of mitochondria protein in sample, gprot/L

10^6 : 1 mol = 10^6 μmol

Appendix I Data

▲ Example analysis

For 20% rat heart tissue homogenate, dilute for 2 times, take 20 μL and carry the assay according to the operation table.

The results are as follows:

the A_1 of the control is 0.597, the A_1 of the sample is 0.722. After 3 minutes, the A_2 of the control is 0.433, the A_2 of the sample is 0.711, $\Delta A_{\text{Control}} = A_1 - A_2 = 0.597 - 0.433 = 0.164$, $\Delta A_{\text{Sample}} = A_1 - A_2 = 0.722 - 0.711 = 0.011$, the concentration of mitochondria protein in sample is 3.75gprot/L, and the calculation result is::

mitochondrial complex I activity (U/gprot)

$$= ((0.164 - 0.011) \div (6600 \times 0.7)) \times 0.24 \div 3 \div 0.02 \div 3.75 \times 2 \times 10^6$$

$$= 70.65 \text{ U/gprot}$$